UNIVERSITY OF GOTHENBURG

Department of Marine Sciences

Biodiversity Assessment in Regenerative Ocean Farms using eDNA Metabarcoding

Student: Mats Heitzmann

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Supervisor: Dr. Marina Panova / Department of Marine Sciences

Examiner: Dr. Ann Larsson / Department of Marine Sciences

Abstract

This study investigates the impact of regenerative small-scale mussel farms on biodiversity in coastal environments using eDNA metabarcoding to assess species richness and community composition. Water samples and Autonomous Reef Monitoring Structures (ARMS) bulk samples were collected from three mussel farms and their corresponding control sites across Sweden and Denmark. Analyses focused on comparing farm and control sites, specifically regarding fish and invertebrate biodiversity, using both COI and 12S markers. The findings suggest that mussel farming may enhance fish species richness within farm sites compared to control areas. Observed biodiversity patterns showed significant overlap between farm and control sites, shaped by local factors, with urban farm sites sharing similar species composition. Despite some limitations, including environmental DNA dispersal and sampling variability, this study provides baseline data and supports eDNA metabarcoding as a valuable tool for biodiversity monitoring in regenerative ocean farming.

Keywords: Regenerative Ocean Farming, eDNA Metabarcoding, Biodiversity, Mussel farming, Aquaculture

Popular Scientific Summary

In response to growing concerns about the sustainability of seafood production, regenerative ocean farming has emerged as an innovative approach that not only provides food but also seeks to restore marine ecosystems. This study focuses on small-scale mussel farms in coastal areas, using environmental DNA (eDNA) to examine their effects on local biodiversity. By collecting and analysing DNA traces from water and special monitoring structures (ARMS units) placed within the farms, the variety of fish and invertebrate species across farm and control sites could be assessed.

The findings suggest that mussel farms may contribute to increased fish biodiversity, as farm sites showed slightly higher numbers of fish species compared to control areas. However, the overall species composition was similar between farm and control locations, indicating that the presence of mussel farming alone does not drastically change local biodiversity. Urban sites like Copenhagen and Helsingborg shared overlapping species profiles, possibly due to similar environmental conditions, while the remote site on Tjörn stood out with a unique community composition.

This research highlights both the strengths and challenges of using eDNA for biodiversity monitoring in open water environments, where factors like water currents and seasonal changes can influence DNA detection. Still, eDNA metabarcoding proved to be an effective tool for detecting a wide range of species with minimal environmental impact. As we continue to seek sustainable seafood solutions, studies like this provide essential insights into the potential ecological benefits of regenerative ocean farms and pave the way for improved biodiversity monitoring techniques in marine farming.

List of Abbreviations

ANOVA - Analysis of Variance

ARMS - Autonomous Reef Monitoring Structures

BRUV - Baited Remote Underwater Video System

CBF - Cool Blue Future

PCoA - Principal Coordinate Analysis

PERMANOVA - Permutational multivariate analysis of variance

ROF - Restorative Ocean Farming

SMURF - Standard Monitoring Units for the Recruitment of Fish

1. Introduction

1.1 The Rise of Aquaculture

In recent decades, the aquaculture sector has expanded faster than any other food production industry (FAO, 2018). From 1961 to 2019, global consumption of aquatic foods grew at an annual rate of 3.0%, nearly double global population growth at 1.6% during the same period (FAO, 2022). By 2020, aquaculture production reached 87.5 million tonnes of aquatic animals, with molluscs, mainly bivalves, accounting for 17.7 million tonnes or about 20% of the total (FAO, 2022). Although these rapid developments hold promise for meeting global food demands, they come with significant sustainability challenges, such as environmental degradation, resource overexploitation, and ecological imbalances, especially in large-scale aquaculture operations (Blanchard et al., 2017; Sampantamit et al., 2020).

1.2 Bivalve Cultivation

Bivalves, cultivated at the sites studied, are emerging as key players in the future of food markets. Their rich nutritional profiles, including high levels of protein, omega-3 fatty acids, vitamin B12, and essential minerals like iron, manganese, and zinc, make them a sustainable choice for healthy diets (Willer & Aldridge, 2020). Unlike terrestrial food sources that require substantial land, water, and fertiliser, bivalve cultivation depends far less on non-renewable inputs, making it a highly sustainable and efficient food production method (Costa-Pierce, 2016). Costello. et al. (2020) predict that by 2050 bivalves such as mussels, clams, and scallops, could account for about 40 percent of global seafood consumption. However, rapid expansion of aquaculture can lead to nutrient pollution, habitat destruction, the introduction of non-indigenous species, biodiversity loss and sediment degradation (Costa-Pierce, 2002; Keeley et al., 2014; Naylor et al., 2000; Piedrahita, 2003; Waples & Drake, 2008). These negative impacts, often associated with aquaculture systems producing finfish like salmon farming (Keeley et al., 2014), can also occur in intensive mussel farming. For example, in the Danish Skive-Fjord, sedimentation of mussels (pseudo-) feces leads to organic enrichment of sediments and potentially induce hypoxic conditions (Holmer et al., 2015).

1.3 Sustainability through Regenerative Practices

Regenerative Ocean Farming (ROF) is an innovative approach in marine aquaculture, focusing on sustainable practices that not only produce seafood but also actively contribute to the restoration of marine ecosystem health (Carr, 2021). ROF differs from traditional aquaculture in several ways: it actively involves the community, utilises resources efficiently, without requiring extra inputs, and consistently leverages the natural services provided by the ecosystem (Smith, 2019). Another key aspect is “scaling out” rather than “scaling up” resulting in multiple small-scale farms rather than a few large-scale monocultures (Cool Blue Future, 2024). By utilising ocean space efficiently and employing multi-trophic systems that mirror natural ecosystems, ROF offers many ecological benefits (Alleway et al., 2023). These benefits include the reduction of nitrogen levels in local water bodies, which is essential for maintaining water quality (Krause-Jensen & Duarte, 2016), as well as enhancing biodiversity through habitat restoration (Carr, 2021).

1.4 Biodiversity

Biodiversity is described as the variety of all different life forms on Earth and it represents the complexity of ecological interactions (Gaston & Spicer, 2003).

Alongside the growing population, another major challenge of our time is the increasing loss of biodiversity primarily driven by changes in land and sea use, direct exploitation of organisms, climate change, pollution, and alien invasive species (IPBES, 2019). The construction of aquaculture farm sites can contribute to the destruction of natural habitats, farmed non-native species can escape and outcompete or interbreed with native species, and large-scale intensive aquaculture can spread diseases and parasites to wild populations (Ahmed et al., 2018; Assefa & Abunna, 2018; Grosholz et al., 2015).

There is growing evidence that biodiversity is essential for supporting ecosystem services, with greater species diversity leading to more stable ecosystems (Worm et al., 2006; Tilman et al., 2006). This makes marine biodiversity crucial for future aquatic food production and maintaining water quality levels (Worm et al., 2006).

In nature, bivalve reefs are recognized as biodiversity hotspots, exhibiting significantly higher biodiversity compared to surrounding areas (Bruno et al., 2003; Johnson, 2020 in Smaal et al., 2019). According to Smaal et al. (2019), this increase in biodiversity and associated ecosystem services is not limited only to natural bivalve reefs but is also observed in aquaculture communities.

1.5 Previous Research and Gap Analysis

Previous studies found that added structure to the water column through aquaculture activities allows the development of diverse communities which are similar to natural reef communities (Alleway & Jeffs, 2023, Callier et al., 2018). Callier et al. (2018) further explain that suspended cultures provide habitats for a diverse range of species, including infaunal and epibenthic organisms, hard substrate species, and offer shelter and refuge from predators for mobile epibenthos. Both the physical infrastructure, such as anchors, buoys, and ropes, and the bivalve populations themselves serve as substrates for numerous species (Murray et al., 2007; Ysebaert et al., 2009). Jansen et al. (2011) studied the settlement of various ascidian, polychaete, and crustacean genera on suspended mussel ropes, reporting a significant increase in species richness over the course of an annual cycle. Additionally, Lutz-Collins et al. (2009) found that community composition to be influenced by cultivation duration. Some of the identified species may compete with cultivated mussels (Lesser et al., 1992), making the promotion of biodiversity in aquaculture appear counterintuitive from a commercial perspective (Dürr & Watson, 2010). However, certain species, such as amphipods and polychaetes, can actually be beneficial by removing mussel faeces and pseudofeces (Kaiser, 2001). This activity ultimately enhances mussel growth and resilience to invasive species like sea squirts (Stachowicz et al., 1999).

Many recent studies on biodiversity assessments in aquaculture farms focused on benthic mobile and infaunal biodiversity using e.g. Baited Remote Underwater Video (BRUV) techniques (e.g. Mascorda-Cabre et al., 2024) or sediment grab samples (Sanchis et al., 2021, Wilding & Nickell, 2013) and others were using manual identification of species on cultivation ropes (Lutz-Collins et al., 2009). Only a few studies so far looked at the pelagic species composition in and around ROF. One of them is a recent study performed by Underwood & Jeffs (2023) in New Zealand who investigated the settlement and recruitment of fish in mussel farms using Standard Monitoring Units for the Recruitment of Fish (SMURFs). They found that fish settlement in aquaculture and natural habitats was equivalent. Another explorative study from the Netherlands assessed the fauna in seaweed farms by eDNA Metabarcoding using water samples and settlement plates (Bernard et al., 2019). Many different taxa were identified, but no control locations were included in the study design.

1.6 eDNA Metabarcoding

eDNA metabarcoding enables enables biodiversity assessment without disturbing the environment. By analyzing water samples, a comprehensive list of taxa can be generated without the need to identify individual organisms (Bernard et al., 2019). This method offers numerous advantages, such as detecting cryptic, rare, or juvenile species that are often difficult to identify morphologically (Bernard et al., 2019). It is also cost-effective and allows species detection with minimal fieldwork (Deiner et al., 2017). Additionally, it is non-invasive, eliminating the need to capture or disturb organisms (Rees et al., 2014), and highly sensitive, making it capable of identifying low-density populations that traditional methods might miss (Thomsen & Willerslev, 2015). Despite its strengths, eDNA metabarcoding has limitations, which are examined in detail in the discussion section.

1.7 Aims and Objectives

This study aims to assess whether eDNA metabarcoding, using water samples and ARMS (Autonomous Reef Monitoring Structures) bulk samples, is an effective method to evaluate fish and invertebrate biodiversity in small-scale regenerative ocean farms (ROF) cultivating blue mussels. In addition to farm sites, control locations were included to enable comparisons of taxa. The central hypothesis is that the habitat restoration effect associated with these farms will result in:

(I) increased species richness and

(II) changes in species composition compared to control sites.

There is ongoing debate about where to draw the line between large-scale intensive aquaculture and ROF. This study focuses exclusively on small-scale (community) farms aligned with the ROF manifesto (Cool Blue Future, 2024), published by the Cool Blue Future initiative, outlining standard principles for Restorative Ocean Farming in Europe. In this report, ‘"Blue Community Gardens” and “Restorative Ocean Farming (ROF)” are used synonymously.

2. Materials and Methods

2.1 Study Design

The first water samples (W1) were taken in February/March 2024 which was combined with the installation of the ARMS units. The second water samples (W2) were taken in July/August 2024 alongside the ARMS retrieval and bulk sample collection (ARMS). Three control groups, one for each site, were included in the study, which results in a total of 6 different surveyed locations.

2.2 Sampling Sites

The farms were chosen based on proximity to Gothenburg and existing contacts with the farms. Control locations were chosen based on similarity to the farm location and feasibility. The sites include (1) the blue community garden in Helsingborg - Havskolonin i Helsingborg, (2) Havhøst - Bølgemarken in the city of Copenhagen and (3) Stigfjordens andelsodling on the Island of Tjörn (Figure 1). Farms 1 & 2 are situated in urban environments, whereas farm 3 is located more remotely in an undisturbed location. The urban farms have a similar size and count of mussel socks (26 in Helsingborg and 94 in Copenhagen) and the cultivation happens on a floating raft surrounded by jetties and other constructions. The farm at Tjörn is located in open water and slightly larger, with containing 50 mussel socks and 60 mussel ropes at each of the two rafts. Each mussel sock contains approximately four kg of mussels and each mussel rope is equivalent to 40 kg of mussels. The Helsingborg control group is located at the Helsingborg Yacht Club, while the Copenhagen control group is positioned at the Green Island floating docks. The Tjörn control group is set in open water, approximately 300 meters from the farm.

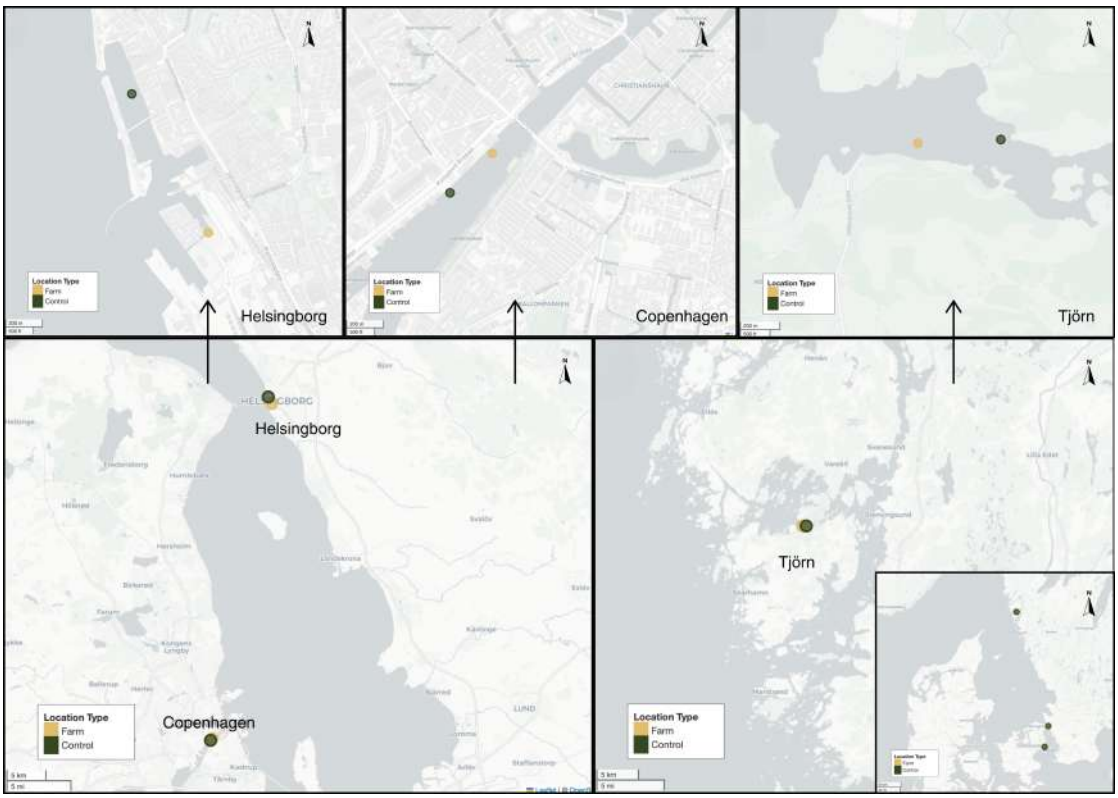

Figure 1: Map of sample locations for farm and control sites (Source: OpenStreetMap contributors, 2024).

2.3 ARMS Deployment, Retrieval and Extraction

For statistical robustness, ARMS units were deployed in triplicates at most locations, with doubles used in certain locations. Each unit was suspended at approximately two meters depth within the mussel farms, and secured by ropes attached to the farm infrastructure. This deployment method was replicated across all study sites, including control locations, to ensure consistency and reliable comparisons. At the control site at Tjörn, where no floating structures were available, a buoy anchored by a custom-made weight was used to keep the ARMS units suspended at the same two-meter depth.

In total, 14 ARMS units were deployed across three farms and their corresponding control sites: three units at the Helsingborg farm (HEL-F), two at the Helsingborg control site (HEL-C), two at the Copenhagen farm (CPH-F), two at the Copenhagen control site (CPH-C), three at the Tjörn farm (TJÖ-F), and two at the Tjörn control site (TJÖ-C).

Each ARMS unit was retrieved after about five months. To avoid contamination during retrieval, gloves were worn at all times, and no plate came into contact with bare skin. All equipment was sterilised with bleach and rinsed thoroughly before and after ARMS processing.

Upon recovery, ARMS units were immediately placed in sterilised tubs (Figure 2). The units were then disassembled, and each plate was photographed as a backup, though the photos were not analysed. Subsequently, all organisms on the plates were scraped off using a metal scraper and homogenised with a mortar and pestle. About 3 ml of the homogenised matter was then transferred into 15 ml Falcon tubes and preserved in 99% ethanol. All samples, including water samples and ARMS samples, were kept in a mobile cooler during fieldwork and transport, and subsequently transferred to a -20°C freezer within 8 hours to minimise DNA degradation (Howlett et al., 2014).

In total, 42 samples were obtained, comprising three replicates from each of the 14 ARMS units. During all lab work, clean personal protective equipment (PPE) was worn, gloves were changed regularly, and the working environment, including pipettes, was frequently decontaminated with bleach and DNAaway. For the DNA extraction of the bulk samples the Quick-DNA™ Fecal/Soil Microbe 96 Kit from Zymo Research was used according to the manufacturer's protocol. The resulting eDNA eluate was stored at -20°C until further analysis.

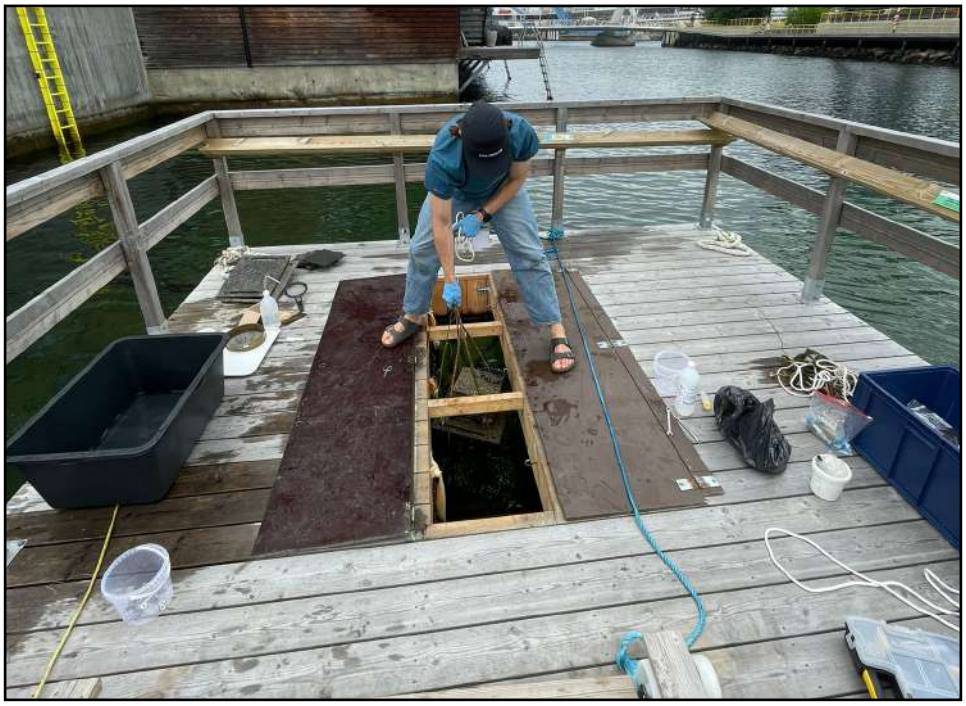

Fig.2: Retrieval setup of the ARMS units in Helsingborg, July 2024

2.4 Water Sample Collection, Filtration and Extraction

For water sample collection, a 1.5 L Niskin water sampler was used to collect water at a depth of two meters. At the farm sites, water was sampled directly between the vertically hanging mussel socks, while at the control sites, it was sampled between the installed ARMS units. A total of 1000 mL was transferred from the water sampler into pre-sterilised buckets for each sample, and sterile 60 mL Luer lock syringes were used to filter the water through 0.45 µm Millipore Sterivex™ cartridge filters. The 1000 mL volume was chosen based on the manufacturer's recommendations and existing eDNA sampling protocols (Cowart et al., 2022).

At each site, an additional filter was used as a field control, where 100 mL of MilliQ water was filtered to monitor for contamination. After filtration, the filters were sealed with parafilm and Luer caps, filled with 2 mL of 99% ethanol to preserve the DNA (Marquina et al., 2021), and stored in sterile Falcon tubes. Instruments were sterilised on-site using 10% bleach, and gloves were changed between processing each sample to avoid cross-contamination.

Triplicates were collected at each location without pooling, resulting in 18 filtered water samples per sampling event, and a total of 36 filtered water samples across the study, excluding the field controls.

For DNA isolation from water samples, the NucleoSpin® eDNA Water kit (Macherey-Nagel GmbH & Co. KG) was used according to the manufacturer's instructions. The resulting eDNA eluate was stored at -20°C until further analysis.

2.6 Metabarcoding of eDNA Samples

Two different markers were used to prepare the amplicon libraries: COI with universal invertebrates primers mlCOIintF and gHCOI2198 (Leray et al., 2013), and 12S rDNA with universal ray-finned fish primers MiFish-U-F, MiFish-U-R (Miya et al., 2015, modified after Stoeckle et al, 2024). The libraries were prepared in two PCR steps to amplify the target gene in the first step, and in the second step to amplify the first PCR product with the Nextera index primers to add Illumina sequencing adapters and dual–index barcodes.

Each COI PCR was performed in 30 µL total volume containing 3 µl KaPa Buffer A 10x, 0,6 µl MgCl2 25 mM, 1,8 µl of 10 mM forward and reverse primers, 0,8 µl dNTP 10 mM, 0,9 µl KaPa Taq 5U/ul, 15 µl H2O, 0.6 µl of bovine serum albumin 20 mg μL^{-1}, and 5 µL of DNA template. Thermocycling conditions for COI involved initial de-

naturation at 95°C for 5 min followed by 95°C for 10 s, 62°C for 30 s (-1° per cycle), and 72°C for 1 min at 16 cycles and 95°C for 10s, 46°C for 30s and 72°C for 1 min at 24 cycles and a final extension at 72°C at 7 min.
Each 12S PCR was performed in 25 µL total volume containing 12.5 µl KaPa HiFi HotStart ReadyMix 2x (Roche), 0,5 µl of 10 mM forward and reverse primers, 0.5 µl of bovine serum albumin 20 mg μL^{-1}, 6 µL H2O, and 5 µL of DNA template. Thermocycling conditions for 12S involved initial denaturation at 98°C for 2 min followed by 40 cycles at 98°C for 40 s, 65°C for 30 s, 72°C for 30 s, and final extension at 72°C for 5 min. PCR products were first visualised on agarose gels to confirm successful amplification, then cleaned using AMPure XP beads (Beckman Coulter) according to the Illumina amplicon preparation protocol.

The second index-PCR with Illumina barcodes was performed in a total volume of 50 µL containing 25 µl KaPa HiFi HotStart ReadyMix 2x (Roche), 5 µL of index 1 and index 2 primers from Illumina Nextera XT v.2 Index kit, 5-15 µL of cleaned inner PCR products and 0-10 µL of PCR-grade water. The volume of water and PCR product was depending on the PCR concentrations which were measured with Qubit fluorometer before and after the second PCR step. Thermocycling conditions for the index-PCR were 95°C for 3 min; 10 cycles at 95°C for 30 s, 55°C for 30 s, 72°C for 30 s; 72°C for 5 min. The final PCR products were cleaned again with AMPure XP beads (Beckman Coulter) and pooled in equimolar concentrations. The sequencing of the samples was performed on the Illumina MiSeq system by Bioinformatics and Data Centre, Core Facilities, Sahlgrenska Academy, University of Gothenburg, Sweden and Clinical Genomics Gothenburg, SciLifeLab in three different sequencing runs.

2.7 Bioinformatic Analyses

For the COI samples, the bioinformatics analyses were performed in R Studio (v. 4.3.1, Posit team, 2023). The raw sequences in FASTQ format were processed using the DADA2 pipeline (Callahan et al., 2016). Primers from both forward and reverse reads were removed with Cutadapt 4.9 with Python 3.11.5 (Martin, 2011). The quality of the forward and reverse reads was assessed by visualising quality profiles of a subset of the data, and read ends with a quality score below 30 were trimmed. During this filtering step, any reads containing ambiguous bases were also removed. An error model was then trained, resulting in a good fit between the observed and ex-

pected errors. Next, de-replication and inference of Amplicon Sequence Variants (ASVs) were performed, followed by denoising of the reads. Forward and reverse reads were merged using the *mergePairs()* function with a minimum overlap of 20 base pairs and no mismatches allowed. Chimeras were removed using the “consensus" method with the *removeBimeraDenovo()* function, and singletons were discarded to maintain data quality. W2 and ARMS samples demonstrated a better trimming performance than W1, with a higher percentage passing the filter (+12,49%) and no discarded reads, which was expected based on the provided sequencing report.

Taxonomic classification was conducted using the RDP Classifier (Wang et al., 2007) against the MIDORI2 COI database. A confidence threshold of 0.7 was applied manually following the integration of the taxonomic output with the sequencing tables generated in DADA2. ASVs that did not meet this confidence threshold or had no taxonomic match were excluded from further analysis. Additionally, samples identified in the negative control were carefully removed from the dataset to prevent potential cross-contamination. The COI taxonomy list was subsequently filtered to include only invertebrates and chordates, allowing for a more targeted analysis.

For the 12S samples, the MiFish pipeline (Zhu et al., 2023) was used, which processes paired-end FASTQ files and performs quality checks with FastQC (Chen et al., 2018), paired-end read assembly with FLASH (Magoč & Salzberg, 2011), and primer removal with Cutadapt (Martin, 2011). Read denoising, chimera removal, and OTU detection were performed using USEARCH (Edgar, 2010). After clustering identical sequences, BLASTN searches were conducted using BLAST+ version 2.9.0 (Camacho et al., 2009) against the reference fish sequence database, which contains 11,064 fish species in the latest version (v. 4.0.5, 2024). The identity threshold was set at 97%. The MiFish pipeline provides three confidence scores as outputs: Confidence, Identity (%), and Confidence score. Reads with a Confidence lower than MODERATE were discarded, which is equivalent to the 70% threshold of the MIDORI2 database. The negative lab control was carefully examined and species that appeared were removed from the respective samples. Contaminants, such as Homo sapiens and Canis lupus, were filtered out.
13 of the W1 samples had sequencing errors and five didn’t pass the read length filter. It was therefore decided to discard all samples from W1 (first water samples) and to focus on W2 and ARMS.

2.8 Statistical Analysis

All analyses were performed in R Studio (v. 4.3.1, Posit team, 2023) using dplyr and vegan packages (Wickham et al., 2023, Oksanen et al., 2022).

In this study, “treatment” refers to the comparison between farm and control locations, with farm sites representing the presence of aquaculture activity and control sites serving as reference points without such activity.

To evaluate whether the treatment affected species richness, a one-way ANOVA was performed separately for each sampling method (W2 and ARMS) across the two datasets (12S and COI). This approach provided detailed insights into both taxonomic groups and sampling methods. The data structure included the number of species found for each replicate at each site. Consequently, four distinct one-way ANOVAs were conducted, with treatment as the explanatory variable and species richness as the response variable. A significance level of 0.05 was used to determine statistical differences.

To assess species composition, unique species from each replicate per treatment and location were pooled and compiled into a presence/absence matrix for the COI, 12S, and combined dataset. The *vegdist* function from the vegan package was used to create a Jaccard distance matrix, which served as the basis for the analysis. A PERMANOVA was performed using the *adonis2* function, with 999 permutations to test the significance of differences in species composition between treatments. Additionally, a Principal Coordinates Analysis (PCoA) was conducted using the *cmdscale* function to visualise the species compositions, providing a graphical representation of community variation across samples. As noted in Chapter 2.3, some locations had uneven numbers of replicates due to missing ARMS units. Given the exploratory nature of this study, unbalanced replicates were accepted, and the resulting reduced statistical power is addressed in the discussion.

3. Results

3.1 Species Richness

The results of the first ANOVA (Table 1) on 12S water samples showed a statistically significant effect of treatment on species richness ($F = 4.971$, $p = 0.0405$), indicating a notable difference between farm and control groups at the 5% significance level.

Tab.1 : Results of the one-way ANOVA for 12S water samples (W2), comparing species richness between farm and control locations

Source	Df	Sum of Squares	Mean Square	F-value	p-value
Treatment	1	9.389	9.389	4.971	0.0405
Residuals	16	30.222	1.889		

The bar plot (Figure 3) demonstrates that mean species richness for the control group was 12.6 (SD = 1.59), while the farm group had a higher mean species richness of 14.0 (SD = 1.12).

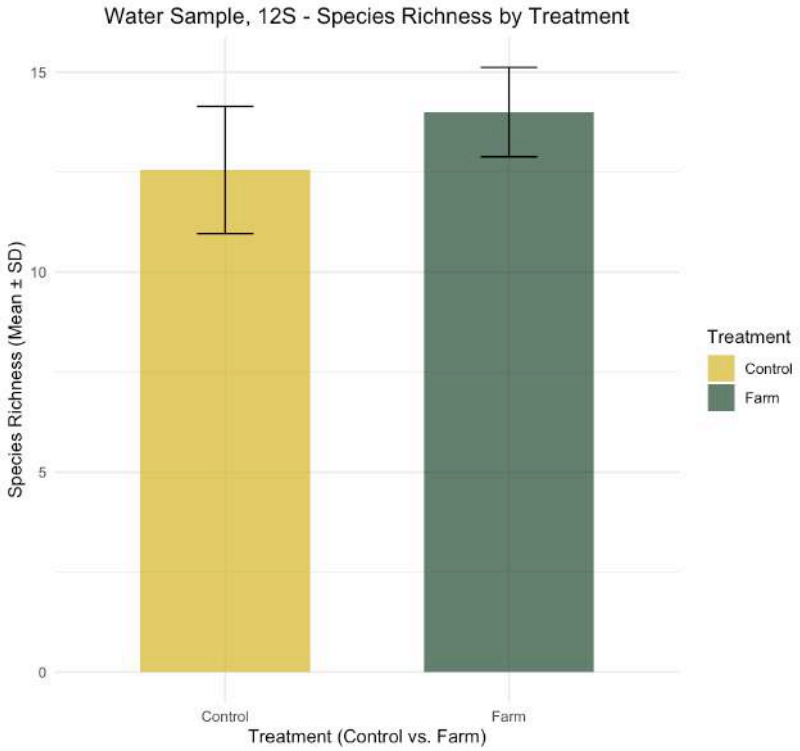

Fig. 3: Bar chart comparing mean species richness between control and farm locations for 12S water samples. Error bars represent the standard deviation (SD), indicating the variability within each group.

The second one-way ANOVA (Table 2) of the W2 COI samples shows no statistically significant effect of treatment on species richness (F = 0.756, p = 0.397).

Tab.2 : Results of the one-way ANOVA for COI water samples (W2), comparing species richness between farm and control locations

Source	Df	Sum of Squares	Mean Square	F-value	p-value
Treatment	1	68.1	68.06	0.756	0.397
Residuals	16	1440.2	90.01		

The bar plot (Fig. 4) shows that the mean species richness was higher in the control sites (12.6) compared to the farm sites (8.67). However, there was substantial variability within each treatment, as indicated by the standard deviations (SD = 10.4 for control and 8.5 for farm).

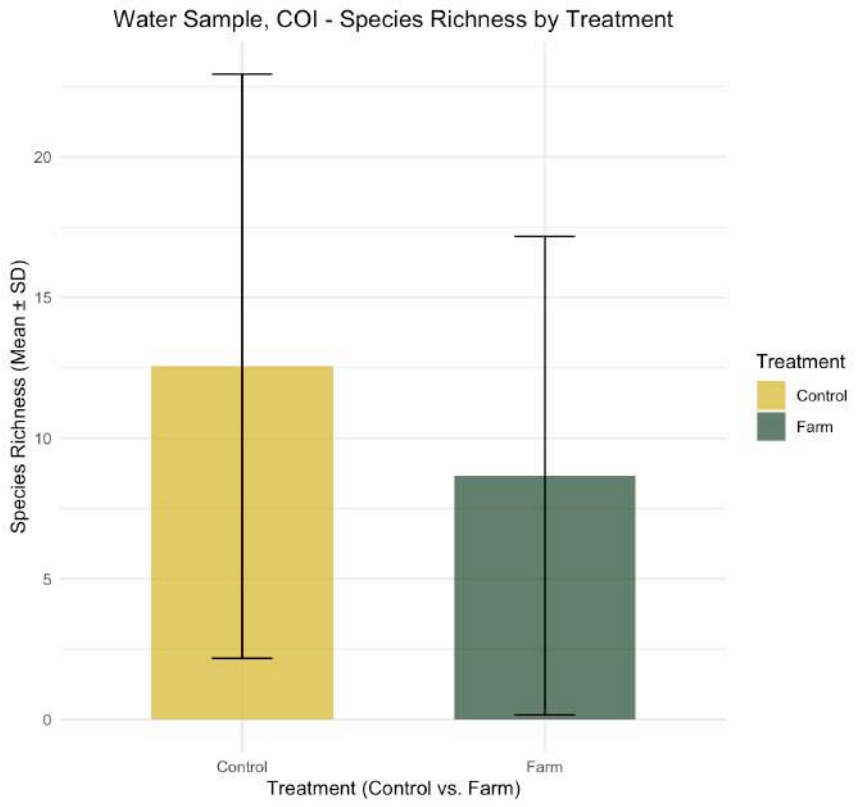

Fig. 4: Bar chart comparing mean species richness between control and farm locations for COI water samples. Error bars represent the standard deviation (SD), indicating the variability within each group.

The third one-way ANOVA (Table 3) was conducted to examine the effect of treatment on species richness within the ARMS 12S samples.

The analysis found no statistically significant effect of Treatment on species richness ($F = 0.086$, $p = 0.774$).

Tab. 3: Results of the one-way ANOVA for 12S ARMS samples (ARMS), comparing species richness between farm and control locations

Source	Df	Sum of Squares	Mean Square	F-value	p-value
Treatment	1	2.4	2.381	0.086	0.774
Residuals	12	331.3	27.611		

The third bar plot (Figure 5) shows that the mean species richness was slightly higher in the control sites (16.3) compared to the farm sites (15.5). However, the standard deviation (SD) values reveal notable differences in variability: the SD for control was 2.58, while the SD for farm was 6.52.

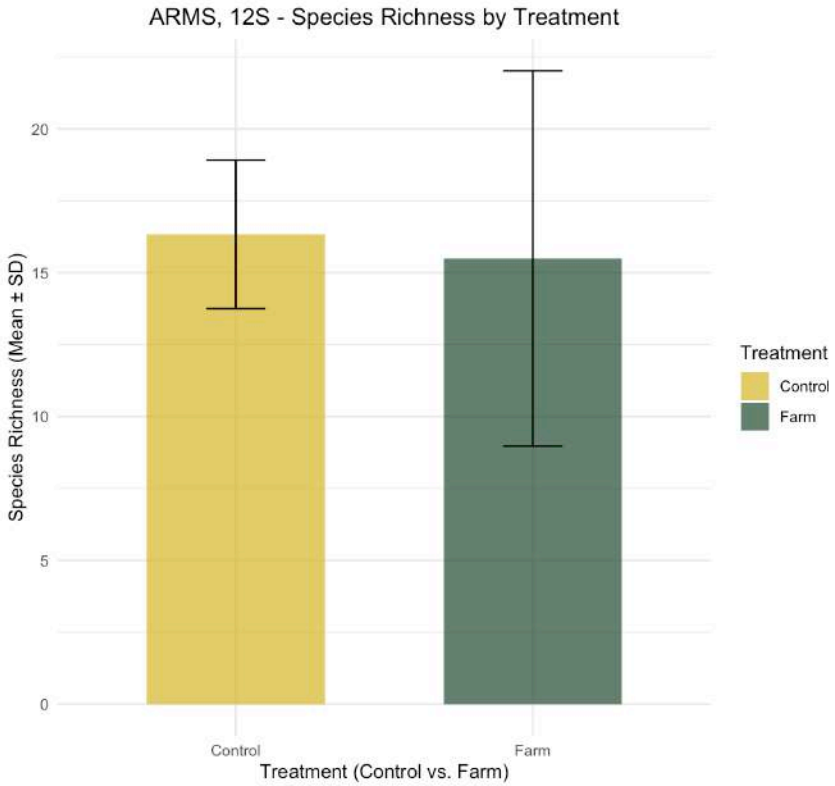

Fig. 5: Bar chart comparing mean species richness between control and farm locations for 12S ARMS samples. Error bars represent the standard deviation (SD), indicating the variability within each group.

The fourth and last one-way ANOVA (Table 4) was conducted to examine the effect of treatment on species richness within the ARMS COI samples. The analysis indicated that there was no statistically significant effect of treatment on species richness ($F = 1.885$, $p = 0.195$).

Tab. 4: Results of the one-way ANOVA for COI ARMS samples (ARMS), comparing species richness between farm and control locations

Source	Df	Sum of Squares	Mean Square	F-value	p-value
Treatment	1	18.67	18.667	1.885	0.195
Residuals	12	118.83	9.903		

The bar plot (Figure 6) illustrates species richness by treatment within the ARMS COI samples. The mean species richness was higher in the farm sites (27.5) compared to the control sites (25.2). The standard deviation (SD) values were 3.13 for control and 3.16 for farm.

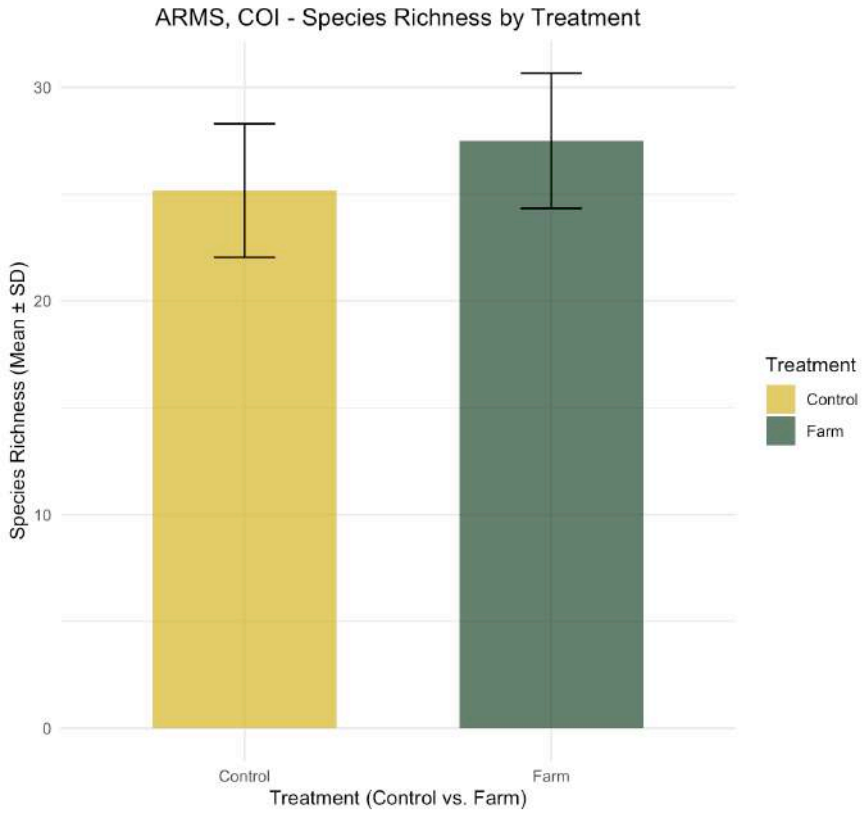

Fig. 6: Bar chart comparing mean species richness between control and farm locations for COI ARMS samples. Error bars represent the standard deviation (SD), indicating the variability within each group.

3.2 Species Composition

PERMANOVA (Table 5) was used to assess the effect of treatment on species composition within the combined 12S samples. The results showed no statistically significant effect ($F = 0.7561$, $p = 0.682$).

Tab. 5: PERMANOVA results for combined 12S samples comparing species composition between farm and control locations using the Jaccard distance metric.

	Df	SumOfSqs	R2	F	Pr(>F)
Model	1	0.0953	0.02458	0.7561	0.682
Residual	30	3.7804	0.97542		
Total	31	3.8757	1.0		

The PCoA plot (Figure 7) provides a visual representation of community composition based on only the 12S data. The distance between points reflects similarity/dissimilarity. The green (farm) and yellow (control) convex hulls cover a fairly broad area, indicating variability within each treatment group.

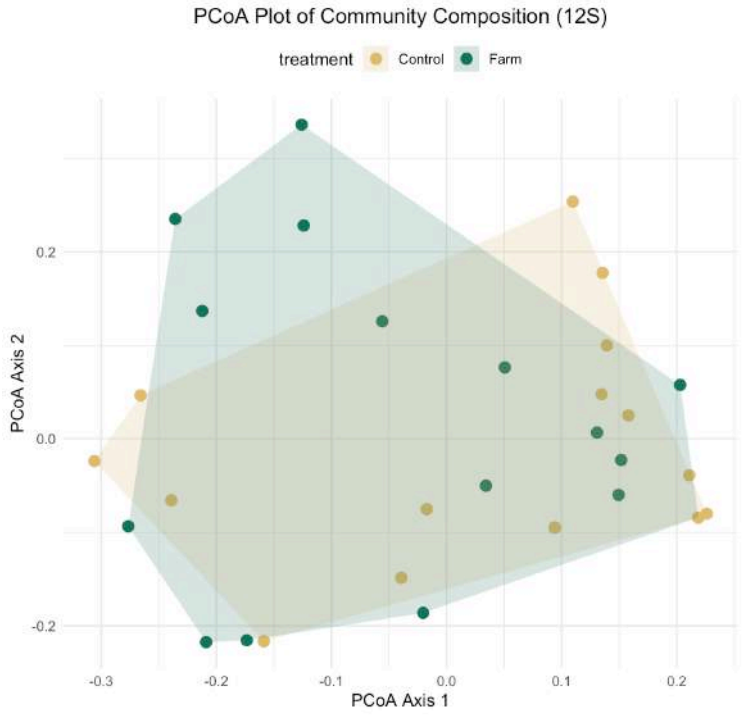

Fig. 7: The plot displays the results of a Principal Coordinates Analysis (PCoA) based on the Jaccard distance metric, illustrating the community composition of 12S samples for both farm and control treatments.

To evaluate the effect of treatment on species composition within the combined COI samples, a second PERMANOVA was performed (Table 6). The analysis indicated no statistically significant differences in species composition between the two treatments ($F = 0.6423$, $p = 0.917$).

Tab. 6: PERMANOVA results for combined COI samples comparing species composition between farm and control locations using the Jaccard distance metric.

	Df	SumOfSqs	R2	F	Pr(>F)
Model	1	0.2274	0.02096	0.6423	0.917
Residual	30	10.622	0.97904		
Total	31	10.8494	1.0		

The COI PCoA plot (Figure 8) shows an overlap in community composition between the farm and control groups, consistent with the PERMANOVA result, which found no statistically significant effect of treatment on community composition.

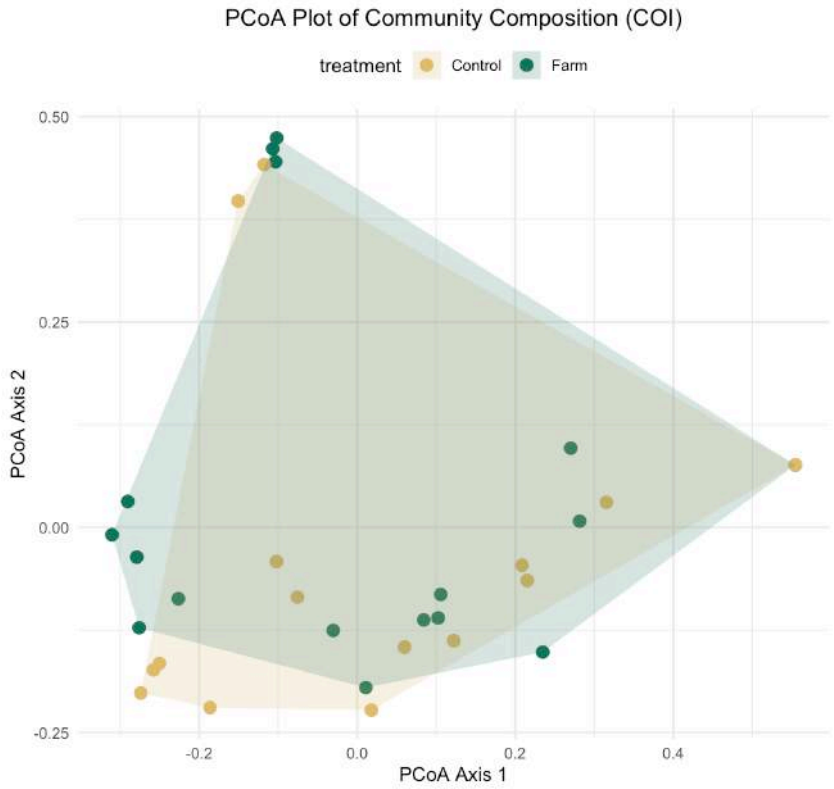

Fig. 8: The plot displays the results of a Principal Coordinates Analysis (PCoA) based on the Jaccard distance metric, illustrating the community composition of COI samples for both farm and control treatments.

The overall results of the PERMANOVA (Table 7) of both data groups (COI & 12S) suggest that there is no statistically significant difference (p = 0.884) in the community composition between farm and control treatments when considering the combined COI and 12S eDNA data.

Tab. 7: PERMANOVA results for combined COI and 12S samples comparing species composition between farm and control locations using the Jaccard distance metric.

	Df	SumOfSqs	R2	F	Pr(>F)
Model	1	0.1537	0.02126	0.6516	0.884
Residual	30	7.0765	0.97874		
Total	31	7.2302	1.0		

The PCoA plot (Figure 9) of the combined COI and 12S data shows the community compositions for both treatments (hulls) and the different locations (dashed ellipses).

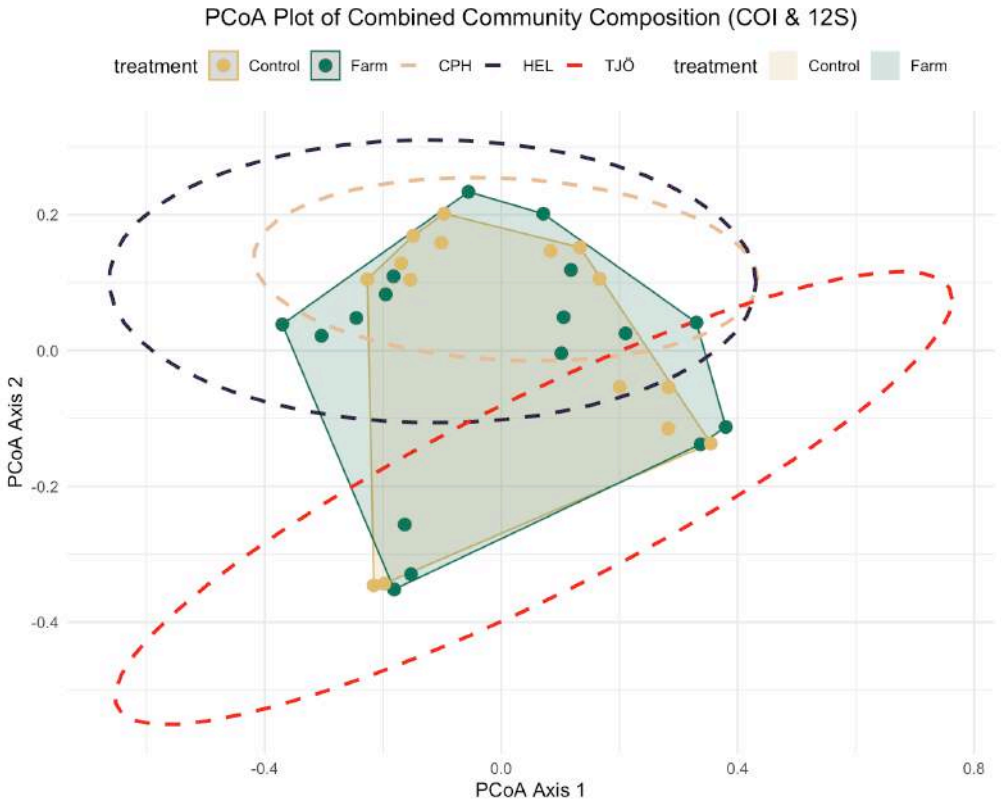

Fig. 9: The plot displays the results of a Principal Coordinates Analysis (PCoA) based on the Jaccard distance metric, illustrating the community composition of COI & 12S samples for both farm and control treatments as well as for the different locations.

3.3 Field Observations

Field observations provided insights into species recruitment on the ARMS units. In Copenhagen, a notable presence of cockles (*Cerastoderma edule*) was observed between the ARMS plates at the farm site, which were absent at the control location. At Tjörn, a high number of sea squirts (*Ciona intestinalis*) settled on the plates, with greater abundance at the control location compared to the farm site. In Helsingborg, sediment between the plates at the control site appeared muddier and darker than at the farm site. Overall, slightly higher biomass was observed on the ARMS units at the farm sites compared to the control sites.

3.4 Discovered Species

The following lists provide an overview of common and unique species that were found. A comprehensive presence-absence matrix for all species combined can be found in the appendix.

Table 8 shows the most common species found at all replicates. No species from the COI dataset were present in all samples.

Tab. 8: Shows the species that were most common across all sites and treatments per replicate

12S - Species present in all samples	**COI**
Aphia minuta (Gobiidae)	*Amphibalanus improvisus (Balanidae)* - Present in 28 samples
Belone belone (Belonidae)	*Polydora cornuta (Spionidae*) - Present in 25 samples
Gobiusculus flavescens (Gobiidae)	*Aurelia aurita* (*Ulmaridae)* - Present in 24 samples
Pholis gunnellus (Pholidae)	*Alitta succinea (Nereididae)* - Present in 22 samples

Table 9 shows the unique species that were found at each treatment for the COI dataset including 25 unique species for the control group and 14 for the farm group.

Tab. 9: Unique species for each treatment in the COI dataset

Control (25 Species)	**COI**	**Farm (14 Species)**	**COI**
Amphitrite figulus	*Aphrodita aculeata*	*Bradysia impatiens*	*Cephalothrix rufifrons*
Arenicola marina	*Caryophyllia smithii*	*Gammarus insensibilis*	*Lasius balcanicus*
Chironomus salincrius	*Chroicocephalus ridibundus*	*Magelona johnstoni*	*Mnemiopsis leidyi*
Colobopyga pritchardiae	*Corymorpha nutans*	*Mycetophila lunata*	*Neochromadora poecilosomoides*
Cricotopus ornatus	*Dermatophagoides farinae*	*Ophiopholis aculeata*	*Palaemon elegans*
Eurytemora affinis	*Flustra foliacea*	*Paratanytarsus dissimilis*	*Psammechinus miliaris*
Gasterosteus aculeatus	*Leuckartiara octona*	*Rhithropanopeus harrisii*	*Spisula subtruncata*
Littorina saxatilis	*Lizzia blondina*		
Obelia geniculata	*Ophiocomina nigra*		
Pleopis polyphemoides	*Polititapes aureus*		
Polydora websteri	*Potamothrix bavaricus*		
Pseudosuberites nudus	*Temora longicornis*		
Testudinella clypeata			

Table 10 shows all unique species per treatment for the 12S dataset including 3 unique fish species for the control group and 9 for the farm group.

Tab. 10: Unique Species per treatment for 12S dataset

Control (3 Species)	12S	Farm (9 Species)	12S
Anguilla anguilla	*Solea solea*	*Centrolabrus exoletus*	*Enchelyopus cimbrius*
Syngnathus rostellatus		*Gadus morhua*	*Lophius piscatorius*
		Pollachius virens	*Pungitius sp.*
		Salmo salar	*Symphodus melops*
		Trachinus draco	

The table below (Table 11) presents the distribution of unique species, summarised across all replicates at each location for each marker (COI on the left and 12S on the right).

Tab. 11: Summary of unique species per treatment and location, total count of species and total count of unique species per treatment

Location/Treatment	Species count (COI)	Location/Treament	Species count (12S)
W2_HEL_F	30	W2_HEL_F	17
W2_HEL_C	41	W2_HEL_C	21
W2_CPH_F	17	W2_CPH_F	22
W2_CPH_C	24	W2_CPH_C	13
W2_TJÖ_F	15	W2_TJÖ_F	19
W2_TJÖ_C	14	W2_TJÖ_C	17
ARMS_HEL_F	39	ARMS_HEL_F	31
ARMS_HEL_C	31	ARMS_HEL_C	23
ARMS_CPH_F	39	ARMS_CPH_F	17
ARMS_CPH_C	28	ARMS_CPH_C	18
ARMS_TJÖ_F	34	ARMS_TJÖ_F	24
ARMS_TJÖ_C	34	ARMS_TJÖ_C	18
COI Total Species	108	12S Total Species	41
FARM UNIQUE	83	FARM UNIQUE	38
CONT UNIQUE	96	CONT UNIQUE	32
Appear in Farm only	14	Appear in Farm only	9
Appear in Cont only	25	Appear in Cont only	3

4. Discussion

4.1 Biodiversity Metrics

This study investigated the effects of regenerative aquaculture practices on species richness and community composition, using eDNA metabarcoding to compare farm and control sites across multiple locations. The findings provide insights into the ecological impact of aquaculture infrastructure, revealing patterns that contribute to our understanding of biodiversity in managed marine environments.

A significant effect was observed in the W2-12S samples when analyzing fish community composition, with a p-value of 0.0405, indicating that species richness was higher at farm sites compared to control sites. The bar plot (Figure 3) shows that, on average, species richness was slightly greater at farm sites, with less variability among the farm samples. Although the p-value confirms statistical significance at the 5% level, suggesting the effect is unlikely due to chance, the F-value of 4.971 indicates a moderate effect size, implying that the difference may not be particularly strong.

The results of the other three ANOVAs (Tables 2-4) indicate no significant difference in species richness between farm and control sites for the W2-COI, ARMS-COI, and ARMS-12S datasets. The high standard deviation in the treatments shown in Figures 3 and 4 suggests that species richness was variable across replicates, with certain sites displaying notably higher or lower richness levels than others.

With overall p-values greater than 0.5, any observed differences in species richness between the treatments are likely due to random variation rather than a true effect of the treatment. This suggests that in those datasets mussel farms had no significant impact on species richness, even though the mean species richness was slightly higher at the farm sites for the ARMS-COI samples (Figure 6).

The PERMANOVA tests could not show any significant difference of species composition between farm and control sites with p-values above 0.5. Overall low R2 values (>0.025) suggests that most of the variation occurs within the treatment groups.

Close observation of the PCoA plots indicates a light asymmetry in community composition between the two treatments for the 12S data. The farm treatment shows a broader range of species, including a greater area absent from control. This could indicate that the farm environment is increasing structural complexity offering shelter and foraging opportunities supporting additional species that do not thrive in the control environment witch agrees with Alleway & Jeffs (2023) and Carbines

(1993). The difference might not be in the overall community composition but rather in the presence of certain species that are more likely to appear in Farm (Table 10). This kind of subtle change might not be picked up well by PERMANOVA because the variability within each treatment group is high, as the R2 value suggests.

In the COI PCoA (Figure 8), the farm samples have a slightly wider spread across the plot, indicating more variability within this group. The control treatment samples are somewhat more clustered, but still overlap significantly with the farm group. The slightly higher variability within the farm group could mean that the treatment allows a greater range of invertebrate community compositions, or it could be due to other environmental factors.

In the overall PCoA plot (Figure 9) the farm group displays greater within-group variability, suggesting that farming conditions may support a broader range of species in general. However, this variability does not translate into a distinct separation from the control group. The overlap suggests that community compositions are largely similar across treatments. The dashed lines representing the locations reveal significant overlap between Copenhagen and Helsingborg, with Copenhagen acting as a subunit within the Helsingborg cluster. In contrast, Tjörn forms a distinct, isolated cluster. This pattern may reflect the urban environments of Helsingborg and Copenhagen, which share similarities, while Tjörn's remote, pristine setting likely supports a different species composition.

The number of unique species per treatment supports the effect of treatment on fish diversity at farm sites (Table 11). Higher numbers of unique invertebrate species in the control group could indicate some filter feeding organisms might compete with the farm mussels, like *Polititapes aureus* or *Pseudosuberites nudus,* and therefore do not appear in the farm treatment (Lesser et al., 1992). The observation of fewer unique filter-feeding species at farm sites (Table 9) supports this hypothesis.

Carbines (1993) describes how fish attracted to farm sites may feed on the community associated with mussel lines, which could also explain the lower number of unique invertebrates at farm sites if fish presence is higher. Overall, the results do not support the hypothesis that ROF practices enhance biodiversity in general, but there is a lead that it could possibly enhance fish biodiversity.

4.2 Methodological Limitations

Alongside the multiple strengths and advantages that eDNA metabarcoding offers, there are also a few limitations, especially in open marine environments.

DNA in well-mixed water systems can spread with currents from distant locations which makes it harder to attribute certain species to the mussel farms themselves (Altermatt et al., 2023). Degradation through UV radiation, temperature, or microbial activity, along with uneven DNA distribution in water bodies, could lead to false negatives (Rees et al., 2014). The contamination risk from external sources in the field and lab is high which can lead to false positives or a large number of species that have to be excluded. Varying shedding and DNA degradation rates among organisms make it challenging to quantify biodiversity using eDNA methods (Elbrecht et al., 2017). Additionally, the number of species detected depends heavily on the selected databases, as they vary in their levels of comprehensiveness (Hajibabaei et al., 2011). Given these challenges, it is important to recognise that eDNA methods have limitations and cannot fully capture ecosystem complexity on their own.

This study design involved three farms, each with its own control site; however, in two cases, the control sites had one fewer ARMS unit, which may have affected the statistical power of the results.

Complex regulatory constraints made it challenging to identify suitable control locations, particularly in urban environments. As a result, some control sites may have been located too close to the farm sites, potentially influencing the results. Additionally, urban harbours already offer various physical structures that promote settlement activity, effectively acting as "mussel farms" themselves. This could have impacted the control group samples, leading to results that do not fully represent a true control scenario.

4.3 Recommendations for Future Research

With only a single set of water samples analysed and a 5-month ARMS deployment, this study offers only a snapshot of the DNA present in the environment. It would be valuable to analyse water samples collected throughout the year to determine if species richness and composition fluctuate with farming activity and to assess whether the removal of physical structures affects local biodiversity.

Although no significant results were found, an interesting observation is the unexpectedly high number of fish species detected across all ARMS samples, suggesting the potential for using these units to monitor fish biodiversity in the future.

Lutz-Collins et al. (2009) suggest that community composition is influenced by the duration of cultivation. Therefore, it would be valuable to investigate biodiversity prior to the establishment of a farm and continue monitoring it over several years. This approach could provide insights into how long-term farming activities affect local ecosystems and species composition.

The visual inspection of ARMS units in the field indicated variations in biomass settlement on the plates. In the future, quantifying this by weighing the biomass could allow for a more detailed comparison between sites.

A closer examination of the identified taxa and their potential roles in the ecosystem would be valuable, particularly by analyzing the presence of red-listed, opportunistic, keystone, or invasive species.

Additionally, a study by Weber et al. (2022) explored molecular diet analysis in mussels, suggesting they could function as natural eDNA samplers. This approach could be a promising method for monitoring biodiversity at mussel farms in future studies.

5. Conclusion

This study concludes that regenerative small-scale mussel farming activities may enhance fish biodiversity within farm areas compared to control sites. However, further research is needed to support this finding and provide a more comprehensive quantification of biodiversity. The study provides baseline data for future investigations and demonstrates that eDNA metabarcoding, with water and ARMS samples, can effectively detect a wide range of taxa in both farm and control environments, making it a valuable supporting method for assessing biodiversity in regenerative ocean farms.

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Final_Combined_Corrected_COI_12S_Presence_Absence

Location	Replicate	Treatment	Acartia bifilosa	Acartia hudsonica	Acartia longiremis	Acartia tonsa	Alitta succinea	Alitta virens	Amphibalanus improvisus	Amphitrite figulus	Aphrodita aculeata	Arenicola marina
HEL	1	Farm	1	0	1	0	1	0	1	0	0	0
HEL	2	Farm	0	0	0	0	0	0	0	0	0	0
HEL	3	Farm	0	0	0	1	1	0	1	0	0	0
HEL	4	Farm	0	0	0	0	1	1	1	0	0	0
HEL	5	Farm	0	0	0	0	1	0	1	0	0	0
HEL	6	Farm	0	0	0	0	1	0	1	0	0	0
HEL	1	Control	0	0	0	0	1	0	1	0	0	0
HEL	2	Control	0	1	0	0	0	0	1	0	0	0
HEL	3	Control	1	0	1	0	1	0	1	1	0	0
HEL	4	Control	0	0	0	0	1	0	1	0	0	0
HEL	5	Control	0	0	0	1	1	0	1	0	0	1
CPH	1	Farm	0	0	0	1	0	0	1	0	0	0
CPH	2	Farm	0	0	0	1	1	0	1	0	0	0
CPH	3	Farm	0	0	0	0	0	0	1	0	0	0
CPH	4	Farm	0	0	0	0	1	0	1	0	0	0
CPH	5	Farm	0	0	0	0	1	0	1	0	0	0
CPH	1	Control	0	0	1	1	1	0	1	0	0	0
CPH	2	Control	0	0	0	1	1	0	1	0	0	0
CPH	3	Control	0	0	0	1	1	0	1	0	0	0
CPH	4	Control	0	0	0	0	0	0	1	0	0	0
CPH	5	Control	0	0	0	0	1	0	1	0	0	0
TJÖ	1	Farm	0	0	0	1	1	0	1	0	0	0
TJÖ	2	Farm	0	0	0	0	0	0	0	0	0	0
TJÖ	3	Farm	0	0	0	0	0	0	0	0	0	0
TJÖ	4	Farm	0	1	0	0	1	0	1	0	0	0
TJÖ	5	Farm	0	1	0	0	1	0	1	0	0	0
TJÖ	6	Farm	0	1	1	0	1	0	1	0	0	0
TJÖ	1	Control	0	0	0	0	0	0	1	0	0	0
TJÖ	2	Control	1	0	1	0	0	0	1	0	0	0
TJÖ	3	Control	0	0	0	0	0	0	0	0	0	0
TJÖ	4	Control	0	1	0	0	1	1	1	0	1	0
TJÖ	5	Control	0	1	0	0	1	1	1	0	0	0

Asterias rubens	Aurelia aurita	Belone belone_x	Bowerbankia gracilis	Bradysia impatiens	Branta canadensis	Carcinus maenas	Caryophyllia smithii	Centropages hamatus	Cephalothrix rufifrons	Cerastoderma edule
0	1	0	0	0	0	1	0	0	0	0
0	1	0	0	0	0	0	0	0	0	0
0	1	1	1	0	0	1	0	1	0	0
0	1	0	0	0	0	0	0	0	0	0
1	1	0	1	0	0	1	0	1	0	1
0	1	0	1	0	0	1	0	1	0	0
0	1	0	1	0	0	1	0	1	0	0
0	0	0	0	0	0	0	0	1	0	0
0	1	0	1	0	0	1	0	1	0	0
0	1	0	1	0	0	0	0	0	0	0
0	1	0	0	0	0	0	0	0	0	1
0	0	0	0	0	0	0	0	0	0	0
0	1	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	1	0	1	1	0	1	0	0	0	1
0	1	0	1	0	0	0	0	0	0	1
0	1	0	0	0	0	0	0	0	0	0
0	0	0	1	0	0	0	0	0	0	0
0	1	0	1	0	0	0	0	0	0	0
0	1	0	1	0	0	0	1	0	0	0
0	1	0	1	0	0	0	0	0	0	0
0	1	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
1	1	0	0	0	0	0	0	0	0	0
0	1	0	0	0	0	1	0	0	1	0
0	1	0	0	0	0	1	0	1	0	0
0	0	0	0	0	0	0	0	0	0	0
0	1	0	0	0	1	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
1	1	0	0	0	0	0	1	0	0	0
1	1	0	0	0	0	0	0	0	0	0

Cerastoderma glaucum	Chironomus salinarius	Chroicocephalus ridibundus	Ciona roulei	Clava multicornis	Clytia hemisphaerica	Colobopyga pritchardiae	Corymorpha nutans	Cricotopus ornatus
0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
0	0	0	0	0	1	0	0	0
1	0	0	0	1	0	0	0	0
1	0	0	0	1	1	0	0	0
1	0	0	0	1	0	0	0	0
0	0	0	0	1	1	0	1	0
0	0	0	0	0	0	0	0	0
1	0	0	0	1	1	0	0	0
1	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
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1	0	0	0	0	0	0	0	0
1	0	0	0	1	0	0	0	0
1	0	0	0	0	0	0	0	0
0	0	0	0	0	0	1	0	0
0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
1	1	1	0	0	0	0	0	1
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0	0	0	1	0	1	0	0	0
0	0	0	1	0	1	0	0	0
0	0	0	1	0	1	0	0	0
0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
0	0	0	1	0	1	0	0	0
0	0	0	1	0	0	0	0	0

Dermatophagoides farinae	Echinocardium cordatum	Emplectonema gracile	Eumida sanguinea	Eurytemora affinis	Evadne nordmanni	Flustra foliacea	Fulica atra	Gammarus insensibilis	Gammarus locusta
0	1	0	0	0	1	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	1	0	1	0	0
0	0	0	0	0	0	0	0	0	1
0	0	0	0	0	1	0	0	0	0
0	0	0	0	0	1	0	0	0	1
0	0	0	0	0	1	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	1	0	0	0	0
0	0	0	0	0	0	0	0	0	1
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0	0	1	0	0	0	0	0	0	0
0	0	1	0	0	0	0	1	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	1	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	1	0	0	0	0	0	0
0	0	1	1	1	0	1	0	0	0

Gammarus oceanicus	Gammarus salinus	Gasterosteus aculeatus	Gobius niger_x	Gobiusculus flavescens_x	Grandidierella japonica	Halichondria panicea	Halocladius variabilis	Harmothoe imbricata
0	0	0	0	1	0	0	0	0
0	0	0	0	0	0	0	0	0
0	0	0	1	1	0	0	0	0
1	1	0	0	1	0	0	1	0
1	1	0	0	1	0	0	1	0
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0	0	0	0	1	1	1	0	0
0	0	0	0	0	0	0	0	0
0	0	0	0	1	1	1	1	0
1	1	0	0	0	1	0	1	0
1	1	0	0	0	1	0	1	0
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0	0	0	1	0	0	0	0	0
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0	1	0	0	0	1	0	0	1
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1	0	0	0	0	0	0	1	1
0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	1
0	0	0	0	0	0	0	0	1

Hediste diversicolor	Idotea baltica	Jaera albifrons	Jassa marmorata	Lacuna vincta	Larus fuscus	Lasius balcanicus	Leuckartiara octona	Liocarcinus depurator	Littorina littorea	Littorina saxatilis	Lizzia blondina
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	1	0	0	0	1	0	1	0	0	0
1	0	1	0	1	0	0	0	1	1	0	0
0	1	1	0	0	0	0	0	1	1	0	0
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0	0	1	0	0	1	0	0	0	1	0	0
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0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	1	0
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1	1	1	0	0	0	0	0	1	1	0	0
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0	0	0	0	0	0	0	0	0	0	0	0
0	0	1	1	0	0	0	0	0	0	0	0
0	0	1	1	0	0	0	0	0	0	0	0
0	0	1	1	0	0	0	0	0	0	0	0
0	0	1	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	1	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	1	0	0	0	0	0	1	0	0	0
0	0	1	1	0	0	0	0	1	1	0	0

Macoma balthica	Magelona johnstoni	Melita nitida	Merlangius merlangus	Mnemiopsis leidyi	Monocorophium acherusicum	Monocorophium insidiosum	Mya arenaria	Mycetophila lunata	Mytilicola orientalis
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0	1	0	0	0	0	0	0	0	0
0	0	0	0	0	0	1	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	1	0	0	0
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0	0	1	0	0	0	1	1	0	0
1	0	0	0	0	0	1	1	0	0
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0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	1	0	0	0	0	1	0	0
0	0	1	0	0	0	1	1	0	1
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0	0	0	0	0	1	1	0	0	0
0	0	0	0	0	1	1	0	0	0
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0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	1	1	0	0	0
0	0	0	0	0	1	1	0	0	0

Mytilus edulis	Mytilus galloprovincialis	Nais elinguis	Neochromadora poecilosomoides	Obelia dichotoma	Obelia geniculata	Obelia longissima	Ophiocomina nigra	Ophiopholis aculeata	Palaemon elegans
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0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
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1	1	0	0	0	0	1	0	0	0

Paracalanus parvus	Paranais litoralis	Paratanytarsus dissimilis	Parvicardium exiguum	Pectinaria koreni	Peringia ulvae	Platynereis dumerilii	Pleopis polyphemoides	Polititapes aureus	Polydora cornuta
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0	0	0	1	0	1	0	0	0	1
0	0	0	1	0	0	0	0	0	1
0	0	0	0	0	1	0	0	0	1
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0	0	0	0	0	0	0	0	0	1
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0	0	0	0	0	0	0	0	0	1
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0	0	0	0	0	0	0	0	1	1
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0	0	0	1	0	1	0	0	0	1
0	0	0	1	0	0	0	1	0	1

Polydora websteri	Potamothrix bavaricus	Psamathe fusca	Psammechinus miliaris	Pseudocalanus acuspes	Pseudosuberites nudus	Pungitius pungitius	Rattus norvegicus	Rhithropanopeus harrisii	Rissoa labiosa
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0	0	0	0	0	0	0	0	0	0
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0	0	1	0	0	0	0	0	0	1
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0	0	1	0	0	0	0	0	0	1
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0	0	1	0	0	0	0	0	1	1
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0	0	1	0	0	0	0	0	0	0
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0	0	0	0	0	1	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	1	0	0	0	0	0	0	0
1	0	1	0	0	0	0	0	0	0

Salmo trutta	Sarsia tubulosa	Scoloplos armiger	Scoloplos armiger	Semibalanus balanoides	Spisula subtruncata	Streblospio benedicti	Syllidia armata	Syngnathus typhle_x	Temora longicornis
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0	0	0	0	1	0	0	1	0	0
0	0	0	0	0	0	0	1	0	0
0	0	0	0	1	0	0	0	0	1
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0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
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0	0	1	0	0	0	1	0	0	0
0	0	0	0	0	0	0	0	0	0
0	1	0	0	1	0	0	1	0	0
0	1	0	0	1	0	0	1	0	0

Testudinella clypeata	Trachurus trachurus_x	Tubificoides benedii	Abramis brama	Anguilla anguilla	Aphia minuta	Belone belone_y	Brosme brosme	Centrolabrus exoletus	Ciliata mustela	Crystallogobius linearis
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0	0	0	0	0	1	1	1	1	0	1
0	0	0	0	0	1	1	1	0	0	0
0	0	0	0	0	1	1	0	0	1	0
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0	0	0	0	0	1	1	0	0	0	0
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0	0	0	1	0	1	1	0	0	0	1
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0	0	0	0	0	1	1	0	0	0	0
0	0	0	0	0	1	1	0	0	0	0
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0	0	0	0	0	1	1	0	0	0	0
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0	0	0	0	1	1	1	0	0	0	0
0	0	0	0	0	1	1	0	0	0	0
0	1	0	0	0	1	1	0	0	0	0

Cyclopterus lumpus	Enchelyopus cimbrius	Engraulis encrasicolus	Gadus morhua	Glyptocephalus cynoglossus	Gobius niger_y	Gobiusculus flavescens_y	Gymnocephalus cernua	Liparis montagui
1	0	0	0	0	1	1	0	0
1	0	0	0	0	1	1	0	0
0	1	0	0	0	1	1	0	0
1	0	0	1	1	1	1	1	0
0	0	0	0	0	0	1	1	0
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0	0	0	0	0	1	1	0	0
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1	0	0	0	0	0	1	0	0
1	0	0	0	0	0	1	1	0
1	0	0	0	1	0	1	1	0

Lophius piscatorius	Micrenophrys lilljeborgii	Microstomus kitt	Neogobius melanostomus	Nerophis ophidion	Pholis gunnellus	Phrynorhombus norvegicus	Pollachius virens	Pomatoschistus microps
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0	0	0	0	0	1	0	0	1
0	0	0	0	0	1	0	0	1
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0	0	1	0	0	1	1	0	1
0	1	1	0	0	1	1	0	0
0	0	1	0	0	1	1	0	0
0	1	1	0	1	1	1	0	1

Pomatoschistus pictus	Pungitius sp.	Pungitius sp. MUSASHI-TOMIYO	Rutilus rutilus	Salmo salar	Scardinius erythrophthalmus	Scophthalmus maximus	Solea solea	Spinachia spinachia	Symphodus melops
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1	0	0	0	0	0	0	0	1	0
1	0	0	1	0	0	0	0	1	0
1	0	0	1	0	0	0	0	0	0

Syngnathus rostellatus	Syngnathus typhle_y	Taurulus bubalis	Trachinus draco	Trachurus trachurus_y	Zeugopterus punctatus
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